The implication of omega-3 polyunsaturated fatty acids in retinal physiology

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Abstract: *Neuronal tissues such as the retina and the brain are characterized by their high content in phospholipids. In the retina, phospholipids can account for until 80% of total lipids and are mainly composed by species belonging to phosphatidyl-choline and phosphatidyl-ethanolamine sub-classes. Within fatty acids esterified on retinal phospholipids, omega-3 PUFAs are major components since docosahexaenoic acid (DHA) can represent until 50% of total fatty acids in the photoreceptor outer segments.*
For long time, DHA is known to play a major role in membrane function and subsequently in visual processes by affecting permeability, fluidity, thickness and the activation of membrane-bound proteins. Today, more and more studies show that PUFAs from the omega-3 series may also operate as protective factors in retinal vascular and immuno-regulatory processes, in maintaining the physiologic redox balance and in cell survival. They may operate within complex systems involving eicosanoids, angiogenic factors, inflammatory factors and matrix metalloproteinases.
This new and emerging concept based on the interrelationship of omega-3 PUFAs with neural and vascular structure and function appears to be essential when considering retinal diseases of public health significance such as age-related macular degeneration.

Key words: *Omega-3 polyunsaturated fatty acids, retina, oxidative stress, inflammation, vasculogenesis*

For a long time, dietary fatty acids have only been considered as the part of the lipid supply necessary for energy supply and tissue growth. The evidence that some polyunsaturated fatty acids (PUFAs) serve as indispensable dietary precursors for biologically active molecules (such as eicosanoids) has given later a greater significance to their study. As a consequence, increasing attention has focused on the functions of omega-3 and omega-6 PUFAs in different organs and particularly in neuronal tissues such as the retina where omega-3 PUFAs are quantitatively important.

Omega-3 PUFAs in retinal structure and function

The structural organisation of the retina

The retina is a thin tissue, which lines the interior of the posterior globe. Since it is embryologically derived from the neural tube, the retina is considered as a peripheral extension of the central nervous system. Histologically, the retina consists in a superposition of different cell layers, in which retinal pigment epithelial cells (RPE), photoreceptor cells (rods and cones), bipolar cells and ganglion cells are of functional importance *(figure 1)*. An anatomical particularity exists between RPE cells and photoreceptors consisting in deep invaginations of photoreceptor outer segments into the extensive apical villous processes of RPE cells, thus creating an intimate physical relationship between these two cell types. The exchange of nutrients and gases between the

retina and the blood is accomplished through two independent circulatory systems. The choroidal system, located between the sclera and RPE cells, consists of an arborized network of capillaries separated from the basal surface of the RPE by the Bruch's membrane. This blood supply serves the outer retinal layers composed by RPE cells and photoreceptors. The second blood supply enters the retina through the optic nerve head and serves as a source of nutrients for all inner retinal layers, including bipolar cells and ganglion cells.

The quantitative and functional importance of omega-3 PUFAs in the retina

Lipids account for about one third of retinal dry weight. The major lipid class within this tissue is represented by phospholipids. Phospholipids can account for until 70% to 80% of total lipids in some animal species [1]. Retinal phospholipids are mainly composed by phosphatidyl-choline and phosphatidyl-ethanolamine whose levels are of 47% and 31% of total phospholipids in human retina, respectively. When regarding to the fatty acid composition of retinal lipids, it appears that over 50% of total fatty acids are unsaturated, of which PUFAs account for 60%. Docosahexaenoic acid (DHA) is the most ubiquitous PUFA in the mammalian retina and is present in up to one third of total fatty acids when considering the whole retina and until up to 60% of total fatty acids within the membrane of the photoreceptor outer segments [1].

The functional significance of this unique fatty acid composition of photoreceptor membranes was extensively studied by the group of Litman and Mitchell [2-6]. These authors have proved that DHA-rich photoreceptor membranes display properties that influence the photon capture by affecting the conformational change of the rhodopsin protein in response to light absorption. Particularly, they have shown that the activation of rhodopsin in response to light was facilitated with increasing unsaturation of the acyl-chain in membrane phospholipids *(figure 2)*. These properties are based on the 22 carbons and the 6 double bonds of the DHA molecule conferring him biophysical and the biochemical particularities that affect membrane fluidity and thickness.

The presence of DHA in mammalian retina appears to be remarkably constant among species and strong conservation mechanisms exist locally and systemically rendering the retinal fatty acid profile resistant to changes by means of dietary manipulation. This is probably why the attempts to deplete mammals such as rat and monkey of retinal omega-3 fatty acids by short- or mid-term dietary manipulations have had limited success [7-12]. Whereas other organs exhibited severe depletion of PUFAs, only minor decreases in retinal PUFAs were observed. In cases of chronic deprivations, retinal DHA losses have been shown to give rise to functional deficits (evaluated by electroretinography) [9, 11, 12] and reduced visual acuity [7, 13].
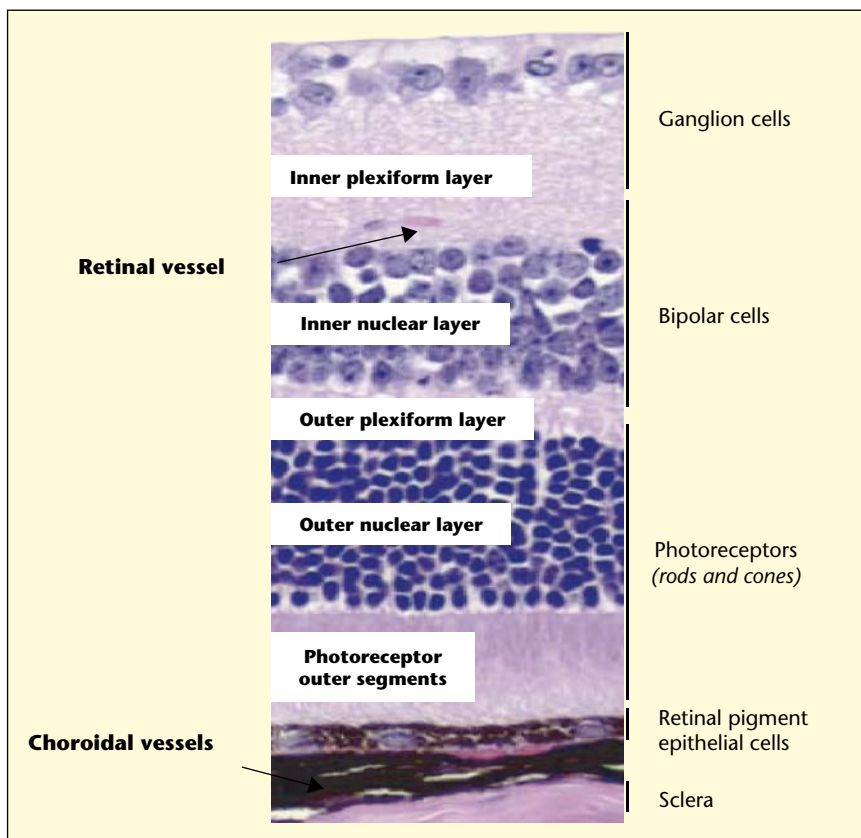
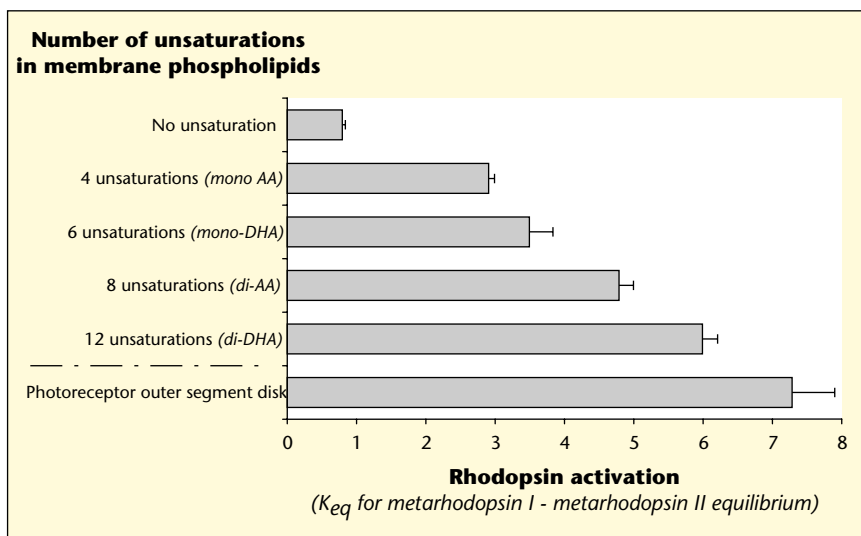Figure 1. *The structural organization of the retina.*

Figure 2. *The effects of membrane-phospholipid unsaturations on the kinetics of rhodopsin activation (adapted from [2]); DHA: docosahexaenoic acid; AA: arachidonic acid.*

The emerging concept about omega-3 PUFAs acting as metabolic bioactivators

For some years, an increasing number of data have proved that omega-3 PUFAs may act as metabolic bioactivators by regulating some key factors involved in cellular processes such as gene expression, oxidative stress, inflammation, cell signaling and apoptosis. Even if all the concerned studies do not directly involve the retinal tissue, these data have built a strong basis for the current and/or future investigations about the functions of omega-3 PUFAs in the retina. The available literature on this topic was extensively reviewed and discussed by SanGiovanni and Chew [14]. Only the major points are presented in this section.

Gene expression

The regulation of gene expression by omega-3 PUFAs can occur at multiple levels. First, omega-3 PUFAs can operate at the transcriptional level by binding to specific ligands that interact with response elements in the promoter region of genes, then affecting gene transcription. This was mainly demonstrated for DHA that can bind nuclear receptors such as the Retinoid X receptor (RXR) and alpha, beta and gamma isoforms of peroxisome proliferator-activated receptor (PPAR) [15–17]. DHA may also act at the post-transcriptional level beyond the synthesis of protein by modifying the gene products formed [18]. Once mRNA is formed, DHA can modify native mRNA processing, mRNA transport, and stability and breakdown rates.

Redox balance and resistance to light damage

From a biochemical point of view, the nature of PUFAs (containing from one to six unsaturations) and their presence into a metabolically active place (exposed to light irradiation and/or high levels of oxygen) would make very probable the formation of oxidized lipids. This appears to be particularly true when considering the high concentration of DHA in retinal photoreceptor outer segments, which are continuously exposed to light illumination. However, the data obtained in vitro and in vivo were very controversial.

In vitro studies on model membranes or liposomes have generally reported a higher susceptibility of PUFAs to peroxidation in response to energy or oxygen exposure. Results from in vivo studies were different. In rats, lower DHA status was associated with lower susceptibility to acute white light exposition [19]. Moreover, rats fed diets deficient in omega-3 PUFAs exhibited better structural outcomes than rats fed alpha-linolenic acid-enriched diet after intense green light illumination [20]. The relationships between omega-3 PUFA or fish intake with reactive oxygen species biomarkers was studied in human. In some cases, in vivo oxidation of was not modified as a function of PUFA intake [21, 22] whereas it was decreased in others [23]. Omega-3 PUFA intake at very high doses was shown to operate as a pro-oxidant [24].

Inflammation

The potential implication of omega-3 PUFAs in the regulation of inflammatory processes was the subject of numerous studies (reviewed by [25]). PUFAs with 20 carbons are precursors of biologically active mediators named "eicosanoids". Eicosanoids are composed by leukotrienes (LTs), prostaglandins (PGs) and thromboxanes (TXs). Eicosanoids formed from omega-3 PUFAs are derived from eicosapentaenoic acid (EPA, omega-3 series) and are from series-5 for LTs and from series-3 for PGs and TXs. Eicosanoids formed from EPA display anti-inflammatory properties. In vitro studies on human cell lines have shown that omega-3 PUFAs (through eicosanoids production) can decrease the expression of TNF-alpha, IFN-gamma IL-1beta, IL-6 and IL-8, the production of IL-2, the expression of surface antigen and the activation and proliferation of T-lymphocytes. Most of these results were confirmed in vivo in animals fed with diets enriched or deprived of omega-3 PUFAs. Even if these results do not directly concern the eye, one can assume that similar cells and/or proteins may be targeted by omega-3 PUFAs during retinal inflammatory response.

Neovascularization

The evident relationships between retinal vascular and neural structures (namely the shared radial orientation of blood vessels and ganglion cell axons and the precise alignment of plexuses with horizontal neurons and astrocytes) is a proof that the retinal vascular anatomy is highly organized. This is probably the reason why retinal neovascularization processes, which are characterized by chaotically oriented and physiologically deficient vessels that do not conform to neuronal organization, are often the cause of loss of vision and eventually blindness in various retinal disorders. Among these disorders, diabetic retinopathy (DR) and age-related macular degeneration (AMD) are the most prevalent in the Western World. For both diseases, adequate therapy is not available to date, laser therapy being performed to physically destroy new vessels and to stop their growing instead of preventing their apparition. For a few years, a promising therapeutic for the prevention of neovascularization processes is based on the administration of anti-angiogenic agents such as inhibitors of vascular endothelial growth factors (VEGFs). These ones were first developed for anticancer medication before being used in eye diseases. However, some important points about the safety or the insufficient selectivity of these agents remain unclear. Consistent evidences suggest that omega-3 PUFAs can exert anti-angiogenic properties through the modulation of processes involved in intracellular signalling, activation of transcription factors and production of inflammatory mediators. The numerous studies demonstrated the involvement of omega-3 PUFAs themselves, that of their secondary metabolites (eicosanoids) or that of the enzymes of their metabolism (cyclooxygenase, lipoxygenase).

The establishment of a functional vascular network requires the regulation of the proliferation, the migration and the differentiation of endothelial cells, the regulation of vascular branching, the regulation of the remoulding of the extracellular matrix in front of the sprouting vessel and the regulation of the stabilization of the nascent blood vessels. Individual studies proved that omega-3 PUFAs are able to i) prevent endothelial cell activation, proliferation, migration as well as their tube forming activity; ii) prevent vascular branching by reducing the expression of adhesion molecules or integrins (such as VCAM-1, E-selectin, ICAM-1); iii) influence tissue remodeling by affecting the activity of matrix metalloproteinases (MMPs); iv) reduce the expression and/or production of pro-angiogenic factors involved in vessel stabilization such as VEGF, TGF-beta, TNF-alpha, FGF, PDGF, angiogenin, angiotensin II, follistatin, IL-8 and leptin (reviewed by [14]).

Cell survival

The rationale for suggesting that omega-3 PUFAs promote cell survival is based on past studies showing that DHA promotes cell survival following various stresses [14]. Recently, it was demonstrated that these properties are not displayed by DHA itself but by one of its metabolites named neuroprotectin D1 (NPD1) (reviewed by [26]). In the retina, the biosynthesis of NPD1 from DHA was demonstrated to occur in RPE cells. In addition to counteract oxidative stress, NPD1 was shown to prevent apoptotic DNA damage by up-regulating the anti-apoptotic proteins from the Bcl-2 family (namely Bcl-2, Bcl-xL, Bfl-1/A1) and down-regulating pro-apoptotic proteins from the Bax family (namely Bax, Bad, Bid, Bik) [27].

Omega-3 PUFAs in human retinal diseases: example of AMD

The biochemical parameters on which omega-3 PUFAs may act are all involved in several retinal diseases that manifest both vascular and neuronal features. Within these diseases, AMD is probably the most relevant since it is the leading cause of vision loss in people aged of more than 65 years in western countries (30% of people over 70 years). The physiopathology of AMD is very complex and not yet fully understood. The current knowledge hypotheses that some unknown events involving oxidative stress and inflammation lead to RPE cell dysfunction in the central area of the retina (macula). This dysfunction is the cause of an accumulation of cell debris and metabolic products in the subretinal area, leading to RPE cell degeneration followed by that of macular photoreceptors. The consequence for the patient is a loss of central vision. In some forms, the retinal tissue attempts to rescue macular cells by promoting choroidal neovascularization, then enhancing the risk of collateral damages such as haemorrhages. The risk factors of AMD include the genetic background (polymorphism of ABCR and ApoE genes), the oxidative stress (aging, smoking, exposition to light, pigmentation) and the nutrient intake including omega-3 PUFAs.

A number of epidemiologic studies were performed to check the prevalence of AMD in relation to omega-3 PUFAs or fish intake. These studies, that were only observational and no interventional, were in accordance by demonstrating a protective effect of omega-3 PUFA consumption regarding the prevalence and the progression of AMD. Within these studies, the results from the Age Related Eye Disease Study (AREDS) show that omega-3 PUFAs decrease the risk of neovascular AMD (progression of AMD) by 40% when considering total omega-3 PUFA intake (OR = 0.61 highest versus lowest quintile; 95% CI: 0.41 - 0.90) and by 50% when considering DHA intake (OR = 0.54 highest versus lowest quintile; 95% CI: 0.36 - 0.80) *(table 1)* [28].

Conclusion

It is now well known that the tissue content in lipids can be modified by the diet. This evidence is also true when considering the relationships between dietary omega-3 PUFAs and retinal fatty acid composition, even some specific strategies to protect this tissue against dietary insufficiencies. Since on the other hand, omega-3 PUFAs are known to be protective against of oxidative stress, inflammation, cell death and abnormal vascularization, one can hypothesize that their consumption would be beneficial against retinal disorders displaying such characteristics. The first epidemiological studies investigating the relationships between dietary fatty acid consumption and ocular pathologies strongly confirm this hypothesis.

Table 1. *Odd ratios for neovascular AMD by energy-adjusted intake of omega-3 PUFAs (adapted from [28]).*

	Quintile	Cases with neovascular AMD	Cases without AMD	OR (95% CI)	*p* trend
Alpha-linolenic acid	1	131	229	1 [reference]	*0.82*
	2	137	220	0.93 (0.67-1.32)	
	3	129	226	0.90 (0.64-1.27)	
	4	127	229	0.96 (0.68-1.36)	
	5	133	211	1.02 (0.72-1.44)	
Eicosapentaenoic acid (EPA)	1	158	208	1 [reference]	*0.05*
	2	146	212	1.02 (0.74-1.43)	
	3	121	228	0.88 (0.62-1.24)	
	4	116	231	0.78 (0.55-1.10)	
	5	116	236	0.75 (0.52-1.08)	
Docosahexaenoic acid (DHA)	1	163	198	1 [reference]	*0.004*
	2	135	213	0.85 (0.61-1.20)	
	3	120	240	0.65 (0.45-0.93)	
	4	131	224	0.75 (0.52-1.08)	
	5	108	240	0.54 (0.36-0.80)	
Total omega-3 PUFAs	1	163	206	1 [reference]	*0.01*
	2	137	207	0.91 (0.65-1.27)	
	3	125	239	0.72 (0.51-1.02)	
	4	121	226	0.77 (0.54-1.10)	
	5	111	237	0.61 (0.41-0.90)	

PUFA: Polyunsaturated fatty acid; AMD: age-related macular degeneration; OR: odd ratio; CI: confidence interval.

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